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Mapping of a thermo-sensitive earliness *per se* gene on *Triticum monococcum* chromosome 1A^m

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Abstract An earliness *per se* gene, designated *Eps-A^{m}1*, was mapped in diploid wheat in F_2 and single-seed descent mapping populations from the cross between cultivated (DV92) and wild (G3116) *Triticum monococcum* accessions. A QTL with a peak on RFLP loci *Xcdo393* and *Xwg241*, the most distal markers on the long arm of chromosome 1A^m, explained 47% of the variation in heading date (LOD score 8.3). Progeny tests for the two $F_{2:3}$ families with critical recombination events between *Xcdo393* and *Xwg241* showed that the gene was distal to *Xcdo393* and linked to *Xwg241*. Progeny tests and replicated experiments with line #3 suggested that *Eps-A^{m}1* was distal to *Xwg241*. This gene showed a large effect on heading date in the controlled environment experiments, and a smaller, but significant, effect under natural conditions. *Eps-A^{m}1* showed significant epistatic interactions with photoperiod and vernalization treatments, suggesting that the different classes of genes affecting heading date interact as part of a complex network that controls the timing of flowering induction. Besides its interactions with other genes affecting heading date, *Eps-A^{m}1* showed a significant interaction with temperature. The effect of temperature was larger in plants carrying the DV92 allele for late flowering than in those carrying the G3116 allele for early flowering. Average differences in heading date between the experiments performed at 16 °C and 23 °C were approximately 11 days ($P < 0.001$) for the lines carrying the *Eps-A^{m}1* allele for early flowering but approximately 50 days ($P < 0.0001$) for the lines carrying the allele for late flowering. The large differences in heading time (average 80 days) observed between plants carrying the G3116 and DV92 alleles when grown at 16 °C, suggest that it would be possible to produce very detailed maps for this gene to facilitate its future positional cloning.

Keywords Earliness *per se* · Diploid wheat · Heading time · QTL mapping · Temperature interaction

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Introduction

Flowering time is important for the adaptability of wheat varieties to different environments, and a complex network of interactions between numerous genes is responsible for the regulation of this important trait. Based on their interactions with environmental signals, these genes can be divided into three broad categories: photoperiod responsive genes (*Ppd*), vernalization responsive genes (*Vrn*), and '*earliness per se*' (*Eps*) genes. Photoperiod responsive genes regulate flowering time in response to day length whereas vernalization responsive genes regulate the requirement of a long exposure to cold temperatures to induce flowerings. The *Eps* genes regulate flowering independently of the previous environmental signals and are usually responsible for the fine-tuning of wheat flowering time.

Variation in heading date observed among completely vernalized wheat varieties grown under inductive photoperiods indicates that there are intrinsic differences in the rate of development between varieties. Even though these differences in flowering time are affected by temperature (Slafer and Rawson 1995), numerous studies have shown that they have a significant genetic component.

Significant differences for days to ear emergence, independently of photoperiod and vernalization, have been observed in hexaploid wheat using a different series of aneuploid and substitution lines in Chinese Spring. Earli-

ness *per se* genes have been reported on wheat chromosomes 2B, 3A, 4B, 4D, 5A, 6B, 6D and 7B (Flood and Halloran 1983; Scarth and Law 1983, 1984; Hoogendoorn 1985; Law 1987; Miura and Worland 1994; Suárez et al. 1995; Worland 1996; Kato et al. 1999).

Genetic studies of *Eps* in wheat have identified some homoeologous regions to barley QTLs on chromosome 5 (Kato et al. 1999) but the small effect of these genes and the complex genetic inheritance of hexaploid wheat have precluded the precise mapping of these genes. A large difference in flowering time that was independent of vernalization and photoperiod requirements was observed in diploid wheat *Triticum monococcum* L. during the characterization of the vernalization genes in this species (Dubcovsky et al. 1998; Tranquilli and Dubcovsky 2000). These preliminary studies showed that when vernalized plants were grown under long-day conditions, the winter genotype (G3116) flowered more than 30 days before the spring genotype (DV92). The first objective of the present study was the precise mapping of the *Eps* genes responsible for this large difference in flowering time in diploid wheat, *T. monococcum*. The second objective was the evaluation of the effect of two different temperatures on the differences in heading dates between the two allelic classes, to improve the experimental conditions for a future high-density mapping project.

Materials and methods

Plant materials

Three accessions of diploid wheat (2n = 14) with different combinations of vernalization genes were used in the present study. All three accessions have a strong response to photoperiod and do not flower under short-day conditions. *T. monococcum* ssp. *monococcum* accession DV92 is a cultivated spring wheat, with recessive alleles at vernalization genes *vrn-A^{m}1* and *vrn-A^{m}2* (Dubcovsky et al. 1998). *T. monococcum* ssp. *aegilopoides* G2528 is a wild spring accession with dominant vernalization alleles *Vrn-A^{m}1* and *Vrn-A^{m}2*. The third accession, G3116, is also a wild wheat from ssp. *aegilopoides* and is the only one within this group with a winter growth habit. This accession carries dominant *Vrn-A^{m}2* vernalization alleles and recessive *vrn-A^{m}1* alleles (Dubcovsky et al. 1998). A genetic model for the action of these two vernalization genes has been proposed in which the role of the *Vrn-A^{m}1* gene is to counteract the *Vrn-A^{m}2*-mediated delay of flowering (Tranquilli and Dubcovsky 2000).

Mapping populations

Three different mapping populations were employed in this study. The first one was the 74 F_2-derived F_3 population from the cross DV92 × G3116 used to develop the RFLP map of *T. monococcum* (Dubcovsky et al. 1996). Only 68 of these families were used in this study because of a limited seed supply. The second one was a 74 single seed-descent (SSD)-line population derived from the DV92 × G3116 F_2 population. The last one was a 102 F_2 population from the cross G2528 × DV92, previously used to study the epistatic interactions between *Vrn-A^{m}1* and *Vrn-A^{m}2* genes (Tranquilli and Dubcovsky 2000).

All lines and F_2 plants from these populations were characterized for their genotypes at the *Vrn-A^{m}1* and *Vrn-A^{m}2* genes by the alleles present at the tightly linked loci *Xwg644* (0.1 cM from *Vrn-A^{m}1*) and *Xucw1* (*Nucellin*) (0.3 cM from *Vrn-A^{m}2*). Nuclear DNAs were isolated from leaves of single F_2 or SSD plants or from pools of 10–20 pooled F_3 plants following the procedure of Dvorak et al. (1988). Southern hybridization was performed as described earlier (Dubcovsky et al. 1994).

The number of days between sowing and emergence of the first spike was scored for each plant. The program Mapmaker QTL (Lander et al. 1987) was initially used to determine the position of the *Eps* gene. Analyses of variance were performed for the critical markers to determine the proportion of variation explained by the *Eps* genes and their degree of dominance. Factorial analyses of variance were used to test the epistatic interactions between the *Eps* and the vernalization genes. Progeny tests of the F_2 plants showing the critical crossover events between the markers more tightly linked to the *Eps* gene were performed to precisely locate the *Eps* gene. Genotypes of each of the progeny test plants were determined for the markers linked to the *Eps* gene to demonstrate if the observed segregation in flowering time was determined by *Eps* or by other genes. Statistical analyses were performed using SAS version 8.0 (SAS Institute 2001).

Experiments

Five different experiments were performed to determine the position of the *Eps* gene in the genetic map, to test its effect in crosses with different genotypes and to evaluate the effect of two different temperatures on the differences in heading dates between the two allelic classes.

Experiment 1

The objective of this first experiment was to map the major genes determining the large difference in heading date observed between vernalized lines G3116 and DV92 grown under long-day conditions. The first mapping experiment was performed using 68 F_3 families from the DV92 × G3116 F_2 population used to construct the RFLP map of *T. monococcum* (Dubcovsky et al. 1996). Five plants from each family and each of the parental lines were vernalized at 5 °C under short-day conditions (8 h of light) for 8 weeks. Plants were then transferred to a greenhouse with temperatures ranging from 15 °C to 20 °C and the natural daylight was extended to 24 h with incandescent light. These conditions were selected to saturate the photoperiod and vernalization requirements in order to observe differences in flowering determined by the *Eps* genes. The number of days between sowing and awn emergence was recorded for individual plants. A QTL analysis was performed using the average heading date values for each F_3 family and the 328 molecular markers available for this population (Dubcovsky et al. 1996).

Progeny tests were performed for $F_{2:3}$ families #58 (20 F_3 plants) and #67 (37 F_3 plants) with critical recombination events between the two RFLP markers most tightly linked to the *Eps* gene in order to map it more precisely. Seedlings from each of these families and from the parental lines were vernalized at 5 °C under short-day conditions for 6 weeks and then transferred to a greenhouse at 20–25 °C, under continuous light. Flowering date and the genotype for the RFLP marker most tightly linked to the *Eps* gene was determined for individual plants.

Experiment 2

The objective of this experiment was to validate the mapping results from Experiment 1 under natural vernalization and photoperiod. It was performed using 74 F_5 SSD individual plants derived from the previous F_2 population (DV92 × G3116). Seeds were vernalized at 5 °C for 20 days and then transplanted to pots located on benches outside the greenhouses during the winter (January 20, Davis, Calif.) for a natural vernalization treatment. Photoperiod varied from short days at planting (9 h and 50 m of light) to long days at the end of the experiment on June 9 (14 h and 47 m of light).

The number of days between planting and awn emergence was recorded for individual plants and analyzed using Mapmaker QTL. Seven molecular markers detecting loci *Xmwg984*, *Xmwg710*, *Xbcd508*, *Xabc261*, *XAga7*, *XksuE11.1* and *Xwg241* on the long arm of chromosome 1A were used for this study. The sources for these markers were previously reported by Dubcovsky et al. (1996).

Experiment 3

The objective of this experiment was to study the epistatic interactions between the *Eps* and *Vrn-A^{m}2* genes. Seventy four F_6 SSD plants derived from the F_5 plants from Experiment 2 (DV92 × G3116) were planted in the greenhouse on August 13, 1998, without vernalization (20–25 °C) and under long-day conditions.

Experiment 4

The objective of this experiment was to determine the effect of the *Eps* gene in a different cross. The genotype for the 102 F_2 plants from the cross G2528 × DV92 was determined with the RFLP marker most tightly linked to the *Eps* gene. Plants were classified by their *Vrn-A^{m}1* and *Vrn-A^{m}2* genotypes into spring and winter classes (Tranquilli and Dubcovsky 2000). The three-way factorial analysis of variance included all possible interactions among the *Eps* gene, *Vrn-A^{m}1* and *Vrn-A^{m}2*. The effect of the *Eps* gene was partitioned into its additive (linear) and dominant (quadratic) effects using orthogonal contrasts. In addition, the same linear and quadratic contrasts were performed separately for the plants with a winter growth habit (genotype *vrn1vrn1/Vrn2-*, 22 plants) and for those with a spring growth habit (genotype *Vrn1-/Vrn2-*, *Vrn1-/vrn2vrn2*, and *vrn1vrn1/vrn2vrn2*; 80 plants). Flowering dates from this F_2 population were available from a previous experiment performed to study the epistatic interactions between *Vrn-A^{m}2* and *Vrn-A^{m}1* (Tranquilli and Dubcovsky 2000). Plants for this experiment were not vernalized and were grown in the greenhouse (20°–25 °C) under continuous light.

Experiment 5

The objective of this experiment was the evaluation of the effect of two different temperatures on the differences in heading dates between the two *Eps* allelic classes. Thirty four homozygous F_6 SSD lines from the DV92 × G3116 cross were selected for this experiment. These lines included all possible combinations of spring and winter types with the two different allelic classes of the *Eps* gene based on the linked RFLP marker. Among the spring SSD lines, eight were *vrn-A^{m}2-late Eps* and ten were *vrn-A^{m}2 early Eps*. Among the winter SSD lines, five were *Vrn-A^{m}2-late Eps* and 12 were *Vrn-A^{m}2 early Eps* Lines were evaluated at two different growing temperatures: 16 °C and 23 °C. For each treatment 15 seedlings from each line were vernalized at 5 °C for 8 weeks under short-day conditions and then transferred to different growth chambers under continuous light in a completely randomized design.

Experiments 1 and 5 were performed at Buenos Aires, Argentina, and Experiments 2, 3 and 4 at Davis, Calif., USA.

Results

Experiment 1. QTL mapping and estimation of the dominance effect

The QTL analysis for the seven chromosomes of *T. monococcum* showed a single QTL for *earliness per se* with a LOD score higher than 3. This QTL explained almost 50% of the variation in heading date for this experiment. Complete vernalization was confirmed by the absence of significant QTLs for heading date at markers completely linked to the *Vrn-A^{m}2* gene.

The peak of the QTL was located on the most-distal region of the long arm of chromosome 1A^m, centered on loci *Xcdo393* and *Xwg241* (LOD score 8.3, Fig. 1A). Analyses of variance for these two markers showed a higher *F* value for *Xwg241* ($F = 28.6$) than for *Xcdo393* ($F = 24.4$). The first locus also explained a larger proportion of the phenotypic variance ($R^2 = 0.47$) than the second one ($R^2 = 0.43$). Orthogonal contrasts for these two loci showed a significant additive effect ($P < 0.0001$) and a non-significant dominant effect ($P = 0.87$) for the *Eps* gene.

Families homozygous for the G3116 *Xwg241* allele (mean time from transplanting to heading = 72 days) flowered 49 days earlier than families homozygous for the DV92 allele (mean time from transplanting to heading = 121 days). This large effect suggested the presence of a major *Eps* gene, designated *Eps-A^{m}1* hereafter. The effect of one dose of the DV92 allele for *Eps-A^{m}1* was to delay flowering for 25 days under the growing conditions of Experiment 1.

Flowering dates of the F_3 families derived from critical F_2 plants #58 and #67 showing recombination between the *Xcdo393* and *Xwg241* loci (Fig. 1B) suggested that *Eps-A^{m}1* was distal to *Xcdo393*. Family #58 flowered 98 days after sowing, exactly as the predicted value for a heterozygous *Eps-A^{m}1*. This family was heterozygous for the *Xwg241* locus and homozygous G3116 (early) for the *Xcdo393* locus (Fig. 1B). Family #67 flowered 126 days after sowing, 5 days later than the average homozygous DV92 *Eps-A^{m}1* plants. This family was homozygous DV92 (late) for the *Xwg241* locus and heterozygous for the *Xcdo393* locus (Fig. 1B).

Progeny tests from plants #58 and #67 confirmed the previous observations. Twenty F_3 plants derived from F_2 plant #58 showed segregation in flowering time. Highly significant differences in flowering time ($P = 0.001$) were detected between these plants when they were grouped by their segregating *Xwg241* genotype. Variation in the *Xwg241* genotype explained 97.4% of the variation in heading date observed in this progeny test. This analysis confirmed that the observed segregation in flowering time was associated with this locus. Based on this result it was concluded that *Eps-A^{m}1* was heterozygous in plant #58, and therefore distal to *Xcdo393* and linked to *Xwg241*.

Progeny tests from F_3 family #67 (*Xcdo393* = heterozygous = H, *Xwg241* = DV92 allele = A) also supported the distal location of *Eps-A^{m}1* relative to *Xcdo393*. No significant differences were detected among the 37 plants from family #67 when they were grouped by the *Xcdo393* alleles ($P = 0.95$) indicating that the *Eps-A^{m}1* gene was in a homozygous state in this family. All three genotypic classes from family #67 showed significant differences in heading date with G3116 ($P < 0.05$) but not with DV92, indicating that plant #67 was homozy-

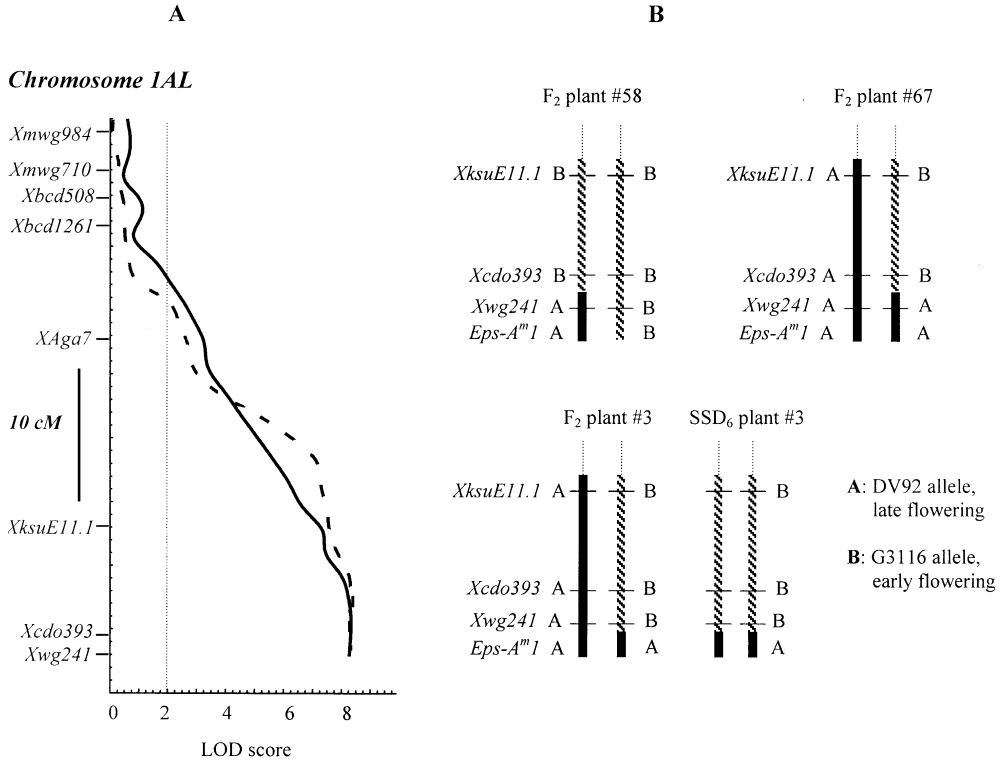

Fig. 1 **a** LOD scores for the QTL analysis of heading date in F_2 (*solid line*) and F_5 SSD (*dotted line*) populations derived from the DV92 × G3116 cross. Map distances are based on the F_2 population. No recombinant chromosomes between *Xcdo393* and *Xwg241* were found in the SSD population. **b** Genotypes of the recombinant F_2 plants #58, #67 and #3 for the *XksuE11.1*, *Xcdo393*, *Xwg241* and *Eps Am1* loci. The non-recombined chromosome was fixed in the F_5 SSD #58 and #67. DV92 and G3116 chromosomes are represented by solid and stripped lines, respectively

gous for the *Eps-Am1* allele from DV92. This result paralleled those from plant #58 and supported the mapping of the *Eps-Am1* gene distal to the *Xcdo393* locus.

A third progeny test was performed for an F_3 family derived from F_2 plant #3 that was heterozygous for all distal markers on chromosome arm 1AmL, but showed a late flowering time (130 days) characteristic of homozygous DV92 plants (Fig. 1B). The 18 F_3 plants analyzed showed a late heading date similar to DV92 independently of the allele present at the *Xwg241* locus. A single-seed descent line derived from F_2 plant #3 showed a similar late heading date in experiment 5 (see results below). This family suggests that *Eps-Am1* may be distal to *Xwg241*.

Experiment 2. Validation of QTL mapping

Highly significant differences ($P < 0.0001$) were detected in heading date between the Single-Seed Descent lines grouped by the *Xwg241* alleles. Lines carrying the DV92 allele flowered an average of 8 days later than those with the G3116 allele. This locus explained 42% of the variation in heading date in this experiment.

Plants grown under natural light and vernalization conditions in this experiment flowered later than those grown under continuous light in the greenhouse in Experiment 1. The difference between plants carrying the G3116 and DV92 alleles was reduced from 49 days in Experiment 1 to 8 days in Experiment 2 (Fig. 2A). These data suggest that when plants have an extended vegetative phase due to a short photoperiod during the early growing stages, the effect of *Eps-Am1* is greatly reduced compared with plants grown under long-day photoperiod conditions.

This experiment was performed with F_5 SSD plants and most of the individuals were homozygous for the *Eps-Am1* locus. However, four plants were still heterozygous for the *Xwg241* locus and they showed an intermediate heading date (127.3 days) between the average of the homozygous DV92 (131.0 days) and G3116 (123.0 days) classes. These results supported the additive effect of *Eps-Am1* observed in Experiment 1.

Mapping of the distal seven RFLP markers from chromosome arm 1AL (Fig. 1A) in the 75 SSD lines, showed the presence of a QTL in the most-distal region of this chromosome. This QTL had a LOD score of 8.3 and the peak of the QTL was centered on RFLP loci *Xcdo393* and *Xwg241*. The chromosomes fixed in the SSD#58 and SSD#67 lines after five generations of self-pollination were those without the crossovers between *Xcdo393* and *Xwg241*. Therefore, it was not possible to distinguish in this experiment which of these two markers was more closely linked to the *Eps-Am1* gene. However, three recombinant individuals between *Xcdo393/Xwg241* and the proximal marker *XksuE11.1* suggested that the *Eps-Am1* gene was distal to *XksuE11.1*. The ANOVA using *XksuE11.1* as the classification variable showed a significantly lower *F* value ($F = 14.4$, $R^2 = 0.31$) compared to the ANOVA using the *Xcdo393/Xwg241* locus as the classification variable ($F = 25.7$, $R^2 = 0.42$). When both loci were tested simultaneously in a 2-way ANOVA using a Type III sum of squares, allelic classes for the *Xcdo393/Xwg241*

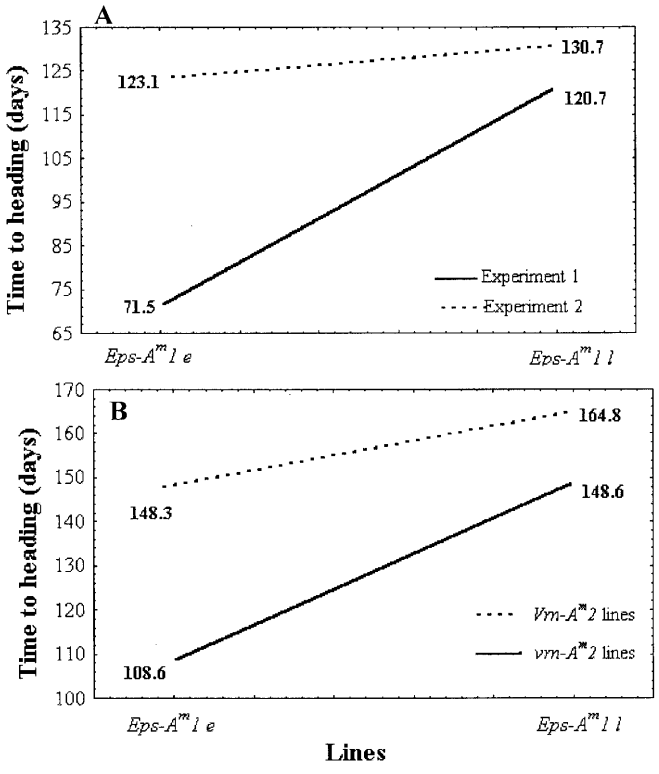

Fig. 2 **a** Comparison of the effect of the *Eps-A^{m}1 e* (allele for early heading time) and *Eps-A^{m}1 l* (allele for late heading time) on average heading date under different environmental conditions. Experiment 1: F_3 families grown under continuous light, Experiment 2: F_5 SSD lines grown under natural light and vernalization conditions after winter sowing. **b** Genetic interaction between *Eps-A^{m}1* and *Vrn-A^{m}2* genes, evaluated in F_5 SSD lines. Heading dates correspond to the average heading date values for each *Vrn-A^{m}2* genotypic class

locus showed significant differences in heading date ($P = 0.002$) but allelic classes for the *XksuE11.1* locus were not significantly different ($P = 0.25$).

Experiment 3. Epistatic interactions between *Eps-A^{m}1* and *Vrn-A^{m}2*

The third experiment used the SSD F_6 seeds from the F_5 lines of Experiment 2 to test the effect of variation in vernalization requirement on the effect of the *Eps-A^{m}1* gene. Genotypes for the *Vrn-A^{m}2* locus were inferred from the completely linked RFLP loci *Xbcd402* and *Xucw1* (*Nucellin*) (Dubcovsky et al. 1998), and those for the *Eps-A^{m}1* gene from the closest locus *Xwg241*.

Highly significant differences in heading date between plants grouped by the *Vrn-A^{m}2* ($P < 0.0001$) and the *Eps-A^{m}1* ($P < 0.0001$) alleles were detected when plants were not vernalized and grown under continuous light. The 2×2 ANOVA showed a significant interaction ($P = 0.02$) between the *Vrn-A^{m}2* and *Eps-A^{m}1* genes and, therefore, the effects of the *Eps-A^{m}1* gene were investigated within each of the vernalization classes.

Within the spring lines (*vrn-A^{m}2*), the average heading date for the plants carrying the DV92 allele at the *Xwg241* locus was 40 days later than for the plants carrying the G3116 allele. However, within the winter lines (*Vrn-A^{m}2*), average heading date for the plants carrying the DV92 allele for the *Xwg241* locus was only 16 days later than for the lines carrying the G3116 allele (Fig. 2B).

Differences in the effect of the *Eps-A^{m}1* alleles between the winter and spring lines parallel the differences observed between Experiments 1 and 2. In both cases the delay of the flowering time (mainly by short-day photoperiod in Experiment 2, and by lack of vernalization in the winter plants in Experiment 3) resulted in a smaller effect of the *Eps-A^{m}1* gene. However, in all cases the differences between the *Eps-A^{m}1* allelic classes was highly significant, suggesting a strong effect of this gene on flowering time (Fig. 2A and B).

Experiment 4. Effect of the *Eps-A^{m}1* gene in a different mapping population

Plants from the G2528 × DV92 population showed significant differences in heading date when they were grouped by their *Vrn-A^{m}1* allele (RFLP *Xwg644*, $P = 0.02$), by their *Vrn-A^{m}2* allele (RFLP *Xbcd402*, $P < 0.01$), or by their *Eps-A^{m}1* allele (RFLP *Xcdo393*, $P = 0.03$). Locus *Xwg241* was not polymorphic in this cross. Significant interactions were detected between the two vernalization genes as reported before by Tranquilli and Dubcovsky (2000).

The effect of the *Eps-A^{m}1* gene was also additive in this cross as indicated by the highly significant linear contrast ($P = 0.01$, Table 1) and the non-significant quadratic contrast for *Xcdo393*. Interactions between the *Eps-A^{m}1* and the vernalization genes were not significant in this study, differing from the results in Experiment 3. This was expected because the additional segregation of *vrn-A^{m}1* in this experiment greatly reduced the proportion of winter plants present in this study (22 winter and 80 spring). However, when the ANOVAs for *Xcdo393* were performed within the individual growth habit classes, the effect of *Eps-A^{m}1* was significant within the spring class ($P = 0.01$) but not significant within the winter class ($P = 0.48$). This result paralleled those from previous experiments and showed that the effect of *Eps-A^{m}1* was large in spring lines with a short growing cycle but was greatly reduced in the winter plants with a very extended growing cycle (average 142 days). The three-way interaction was not significant ($P = 1.00$, Table 1).

Experiment 5. Effect of temperature on the differences between Eps-A^{m}1 alleles

Previous experiments indicated that the best separation between the two alleles was obtained with fully vernali-

Table 1 Three-way ANOVA for heading date based on *Vrn-A^{m}1*, *Vrn-A^{m}2* and *Eps-A^{m}1* allelic classes (Experiment 4). The effect of *Eps-A^{m}1* was further partitioned into its linear (additive) and quadratic (dominant) effects. Additional analyses for the effect of the *Eps-A^{m}1* alleles were performed within the spring and winter plants

Source	df	Type III sum of squares	Mean squares	*F* value	*P*
Vrn-A^{m}1	1	1,135.6	1,135.6	5.84	**0.02**
Vrn-A^{m}2	1	16,593.0	16,593.0	85.31	<**0.01**
Eps-A^{m}1	2	1,432.7	716.4	3.68	**0.03**
Linear	1	1,316.0	1,316.0	6.77	**0.01**
Quadratic	1	14.8	14.8	0.08	0.78
*Vrn-A^{m}1*Vrn-A^{m}2*	1	869.2	869.2	4.47	**0.04**
*Eps-A^{m}1*Vrn-A^{m}1*	2	200.9	100.5	0.52	0.60
*Eps-A^{m}1*Vrn-A^{m}2*	2	66.7	33.4	0.17	0.84
*Eps-A^{m}1*Vrn-A^{m}1*Vrn-A^{m}2*	2	1.3	0.6	0.00	1.00
Eps-A^{m}1, within 80 spring plants					
Linear	1	1,244.2	1,244.2	6.40	**0.01**
Quadratic	1	0.2	0.2	0.00	0.98
Eps-A^{m}1, within 22 winter plants					
Linear	1	96.9	96.9	0.50	0.48
Quadratic	1	104.9	104.9	0.54	0.46
Error term	90	17,505.8	194.5		

zed plants grown under long-day conditions. In this experiment, two temperatures (16 °C and 23 °C) were tested to select the one that better differentiates the alternative alleles and to study the interaction between temperature and the *Eps-A^{m}1* effect on flowering time. Flowering time was analyzed as days to heading.

No significant differences in days to heading were detected between plants grouped by the *Vrn-A^{m}2* alleles. This was expected because the plants were fully vernalized. Therefore, plants were only grouped by their *Eps-A^{m}1* genotype for the following analyses.

The 2 × 2 factorial ANOVA for genotypes and temperatures showed highly significant differences in days to heading for both factors ($P < 0.0001$, Fig. 3). A significant genotype by temperature interaction ($P < 0.0001$) suggested a differential effect of temperature on the two allelic classes of *Eps-A^{m}1*.

Experiments performed at both temperatures showed two clear groups of genotypes, one integrated by early lines carrying the *Xwg241*-G3116 allele and the other formed by late lines carrying the *Xwg241*-DV92 allele (Fig. 3). There was one exception, line SSD #3, a late line carrying the *Xwg241*-G3116 allele (Fig. 3). Results from the 16 °C and 23 °C experiments showed that SSD#3 plants flowered at the same time as the late group with the *Eps-A^{m}1*-DV92 allele, supporting the hypothesis that this line is homozygous for a recombined chromosome with a recombination point between the most-distal marker *Xwg241* and the *Eps-A^{m}1* gene (see also Experiment 1 and Fig. 1B). SSD#3 was included in the late group for the following analysis.

In the 16 °C experiment, the time to heading for the plants carrying the DV92 allele for *Xwg241* (165 days) was 80 days later than for those carrying the G3116 allele (85 days, $P < 0.0001$, Fig. 3). Heading dates for the lines carrying the DV92 alleles varied between 153 and 181 days, whereas heading dates for the lines (except SSD#3) carrying the G3116 alleles varied between 65 and 101 days. The average of ten SSD#3 plants was 162 days.

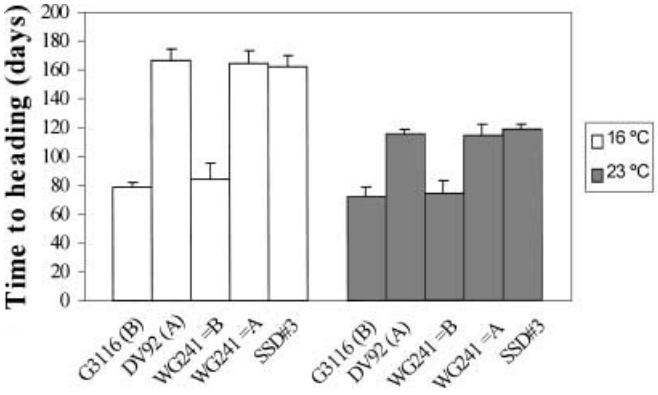

Fig. 3 Average heading dates of F_6 SSD lines grown under 16 °C (*white bars*) and 23 °C (*gray bars*). From left to right the genotypes are: *1* Parental line G3116; *2* Parental line DV92; *3* SSD lines carrying the G3116 (*B*) allele at RFLP locus *Xwg241*; *4* SSD lines carrying the DV92 allele at RFLP locus *Xwg241*; *5* SSD line#3

In the experiment conducted at 23 °C, the time to heading for the plants carrying the DV92 allele for *Xwg241* averaged 115 days, with a range of 99–124 days. This time to heading was 40 days later on average ($P < 0.0001$) than those (except SSD#3) carrying the G3116 allele (average: 74 days, range: 50–94 days). Therefore, differences between the two *Eps-A^{m}1* allelic classes were almost twice as large at 16 °C (80 days) than at 23 °C (40 days).

The effect of temperature was larger in the plants carrying the late DV92 allele than in those carrying the early G3116 allele. Differences between 16 °C and 23 °C for the early lines were approximately 11 days ($P < 0.001$) but for the late lines were approximately 50 days ($P < 0.0001$) (Fig. 3).

Discussion

Mapping

This study provides evidence for the presence of an earliness *per se* gene with a large effect on heading date in diploid wheat *T. monococcum*. Under controlled conditions, plants carrying the different allelic variants for *Eps-A^{m}1* separated into two phenotypic groups with non-overlapping heading dates. The clear segregation of the two allelic classes facilitated the precise mapping of the *Eps-A^{m}1* gene on the distal end of the genetic map of chromosome 1A^m L of *T. monococcum*. Progeny tests of the two $F_{2:3}$ families showing crossovers between the two most distal markers on 1A^{m}L demonstrated that *Eps-A^{m}1* was located distal to *Xcdo393*. Results from the progeny test of $F_{2:3}$ family #3 and the replicated tests of the SSD line derived from this family, consistently suggested that the *Eps-A^{m}1* gene is distal to *Xwg241*, the most distal marker on *T. monococcum* chromosome 1A^m.

Efforts to find markers distal to *Xwg241* in *T. monococcum* were not successful. Locus *XgbxG259* (Mingeot and Jacquemin 1999) was mapped distal to *Xwg241* on chromosome 1A of hexaploid wheat, but the alleles mapped in both the *T. monococcum* F_2 and SSD mapping populations were linked to *XksuE11.1* and proximal to *Xcdo393*. A second locus detected by the KSUE11 probe, *XksuE11.2*, was also mapped distal to *Xwg241* (though at a LOD score < 3) on chromosome 1A of hexaploid wheat (Van Deynze et al. 1995). The five polymorphic bands mapped in the *T. monococcum* SSD mapping population were all linked to *XksuE11.1* (only one faint band was not polymorphic), suggesting that the *XksuE11.2* duplication may be absent in *T. monococcum*. In barley, loci *Xabg55* and *Xbcd351* were mapped distal to *Xwg241* in the cross Galeon × Haruna Nijo (Langridge et al. 1995). Unfortunately, *Xbcd351* was mapped on chromosome 5A^m in *T. monococcum* and the multiple RFLP fragments detected with probe ABG55 were mapped on chromosomes 3A^m, 4A^m, 5A^m and even 1A^m, but 78 cM proximal to *Xwg241* (Dubcovsky et al. 1996). A recent study in tetraploid wheat showed the presence of an AFLP marker 11.5 cM distal to *Xwg241* on chromosome 1A (Nachit et al. 2001), suggesting that it should be possible to find markers distal to *Xwg241*.

Gene order is well conserved between wheat and barley (Dubcovsky et al. 1996), and many genes affecting heading time have been observed in colinear positions in the maps of these two genera. Good examples of this conservation are the vernalization genes *Vrn1* and *Vrn2* (Dubcovsky et al. 1998) and the photoperiod gene *Ppd1* (Snape et al. 1996). There are also examples of earliness *per se* genes in colinear regions on wheat and barley homoeologous groups 2 and 5 (Snape et al. 1996; Worland 1996; Kato et al. 1999). However, comparative analyses of earliness *per se* genes should be considered with caution because most of these genes have been mapped as QTLs covering relatively large regions of the chromosomes. Taking into account this limitation, some QTL studies in barley suggest the possibility of the existence of a barley orthologue to the *T. monococcum Eps-A^{m}1* gene. Significant QTLs for heading date were detected on the distal end of chromosome arm 1HL in the double-haploid mapping populations 'Harrington' × 'Morex' and 'Harrington' × 'TR306' (Tinker et al. 1996; Marquez-Cedillo et al. 2001, http://www.css.orst.edu/barley/nabgmp/qtlsum.htm). In these two barley populations the most-distal marker on 1HL was *Xmwg912*, which maps 8.5 cM proximal to *Xwg241* in chromosome 1A (Van Deynze et al. 1995). It would be interesting to see if the LOD scores for the barley QTLs for heading-date increase for markers located closer to *Xwg241*, and therefore to the putative orthologue of *Eps-A^{m}1*.

Two other QTLs for heading date were detected on the long arm of homoeologous group 1. The first one, responsive to photoperiod and designated *Ppd-H2* (Laurie et al. 1995), was mapped in between RFLP loci *Xpsr162* and *Xbcd304*. The second one was mapped between locus *Xbcd265* and isozyme locus *iPgd* (Pan et al. 1994). However, these two regions are more than 60-cM proximal to *Xwg241* (Dubcovsky et al. 1995) and, therefore, they were most likely determined by genes different from *Eps-A^{m}1*. No earliness *per se* genes have been reported on the distal region of the long arm of homoeologous group 1 in bread wheat, probably due to its complex allohexaploid condition.

Interactions of *Eps-A^{m}1* with environmental factors and vernalization genes

Differences in heading date between genotypes carrying each of the *Eps-A^{m}1* alleles were detected not only under controlled environments, but also under the natural vernalization and photoperiod conditions of winter-sowing outside the green house (Experiment 2). This result suggested that this gene has a strong effect on the determination of heading time in diploid wheat. The large effect of this gene was particularly evident when vernalized plants were grown at a relatively low temperature (16 °C), where the heading date for the plants carrying the *T. monococcum* DV92 allele was an average of 80 days later than the heading date of plants carrying the G3116 allele. Such a large effect has not been observed in the previously reported earliness *per se* gene in the *Triticeae*, that usually results in heading-date differences of just a few days (Flood and Halloran 1983; Scarth and Law 1983; Hoogendoorn 1985; Miura and Worland 1994; Laurie et al. 1995; Worland 1996; Kato et al. 1999).

T. monococcum DV92 differs from all other accessions of *T. monococcum* studied so far by the presence of a recessive *vrn-2* allele (Dubcovsky et al. 1998; Goncharov 1998). The *Vrn-2* gene is dominant for the winter growth habit, and may be involved in the repression pathway of the floral initiation (Tranquilli and

Dubcovsky 2000). It is tempting to speculate that the *Eps-A^{m}1* DV92-allele (conferring late-flowering time) was selected to balance the very early flowering determined by the presence of the recessive *vrn2* allele.

The unusually large effect of the *Eps-A^{m}1* gene, observed under complete vernalization and the long photoperiod, was reduced to a few days when flowering initiation was delayed either by a limiting photoperiod or by the absence of vernalization; nevertheless the effect was still significant ($P < 0.0001$). Significant interactions were observed between the *Eps-A^{m}1* and *Vrn-A^{m}2* genes suggesting that the different classes of genes affecting heading date (*Vrn*, *Ppd* and *Eps*) interact as part of a complex network that controls the timing of flowering induction.

Besides its interactions with other genes affecting heading date, the *Eps-A^{m}1* gene showed a significant interaction with temperature. The DV92 allele (late) was highly influenced by the growing temperature, whereas the G3116 allele (early) showed a smaller difference between the two temperatures tested in this experiment. The differences in heading date between the two *Eps-A^{m}1* alleles and the interaction between this gene and temperature were also significant ($P < 0.0001$) when heading dates were transformed into total thermal time (days × temperature, data not shown).

Other authors have pointed out the existence of an interaction between genotype and temperature on heading date in wheat when vernalization and photoperiod requirements are satisfied (Piratesh and Welsh 1980; Slafer and Rawson 1995). However, these previous studies were based on comparisons of different cultivars that may carry different allelic variants at numerous earliness *per se* loci. This study demonstrated the interactions between a specific earliness *per se* gene with temperature. This was possible by the use of molecular markers tightly linked to the *Eps-A^{m}1* gene and the study of a well-characterized segregating population of diploid wheat.

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References

Dubcovsky J, Galvez AF, Dvorak J (1994) Comparison of the genetic organization of the early salt stress response gene system in salt tolerance *Lophopyrum elongatum* and salt-sensitive wheat. Theor Appl Genet 87:957–964

Dubcovsky J, Luo M-C, Dvorak J (1995) Differentiation between homoeologous chromosomes 1A of wheat and 1A^m of *Triticum monococcum* and its recognition by the wheat *Ph1* locus. Proc Nat Acad Sci USA 92:6645–6649

Dubcovsky J, Luo MC, Zhong GY, Brandsteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L. and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dubcovsky J, Lijavetsky D, Appendino L, Tranquilli G (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97: 968–975

Dvorak J, McGuire P, Cassidy B (1988) Apparent sources of the A genomes of wheats inferred from the polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. Genome 30:680–689

Flood RG, Halloran GM (1983) The influence of certain chromosomes of the hexaploid wheat cultivar Thatcher on time to ear emergence in Chinese Spring. Euphytica 32:121–124

Goncharov NP (1998) Genetic resources of wheat related species: the *Vrn* genes controlling growth habit (spring vs. winter). Euphytica 100:371–376

Hoogendoorn J (1985) A reciprocal F_1 analysis of the genetic control of ear emergence, number of leaves and number of spikelets in wheat. Euphytica 34:545–558

Kato K, Miura H, Sawada S (1999) Detection of an earliness *per se* quantitative trait locus in the proximal region of wheat chromosome 5AL. Plant Breed 118:391–394

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MAPMAKER: an integrated computer package for the construction of primary linkage maps of experimental and natural populations. Genomics 1:172–175

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. Mol Breed 1:389–395

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter ×spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Law CN (1987) The genetic control of daylength response in wheat. In: Averton JG (ed) Manipulation of flowering. Butterworth and Co, pp 225–240

Marquez-Cedillo LA, Hayes PM, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2001) QTL analysis of agronomic traits in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 103:625–637

Mingeot D, Jacquemin JM (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98:1132–1137

Miura H, Worland AJ (1994) Genetic control of vernalization, day length response, and earliness *per se* by homoeologous group-3 chromosomes in wheat. Plant Breed 113:160–169

Nachit MM, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairallah M, Ribaut JM, Tanzarella OA, Porceddu E, Sorrells ME (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L. var. *durum*). Theor Appl Genet 102:177–186

Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karasi I, Bedö Z (1994) Genetic analysis of the components of winter hardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910

Piratesh B, Welsh JR (1980) Effect of temperature on heading date of wheat cultivars under a lengthening photoperiod. Crop Sci 20:453–456

SAS Institute Incorporated (2001) SAS user's guide, version 8. SAS Institute, Inc, Cary, New York

Scarth R, Law CN (1983) The location of the photoperiod gene, *Ppd2*, and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. Heredity 51:607–619

Scarth R, Law CN (1984) The control of the day length response in wheat by the group 2 chromosomes. Z Pflanzenzuchtg 92: 140–150

Slafer GA, Rawson HM (1995) Intrinsic earliness and basic development rate assessed for their response to temperature in wheat. Euphytica 83:175–183

Snape JW, Quarrie SA, Laurie DA (1996) Comparative mapping and its use for the genetic analysis of agronomic characters in wheat. Euphytica 89:27–31

Suárez EY, Worland AJ, Appendino ML (1995) Cromosoma con gran efecto sobre período a espigazón en un trigo argentino "precocidad *per se*". XXVI Congreso Argentino de Genética, Bariloche, Argentina

Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine B, Choo TM, Briggs KG, Ullrich SE, Frankowiank JD, Blake TK, Graf RJ, Dofing SN, Saghai Maroof MA, Scoles GJ, Hoofman D, Dahleen LS, Kilian A, Chen F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Region of the genome that affects agronomic performance in two row barley. Crop Sci 36:1053–1062

Tranquilli G, Dubcovsky J (2000) Epistatic interaction between vernalization genes *Vrn* A^m1 and *Vrn-*A^m2 in diploid wheat. J Hered 91:304–306

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group 1 chromosomes of *Triticeae* species and their relation to chromosomes in rice and oat. Genome 38:45–59

Worland JA (1996) The influence of flowering-time genes on environmental adaptability in European wheats. Euphytica 89:49–57